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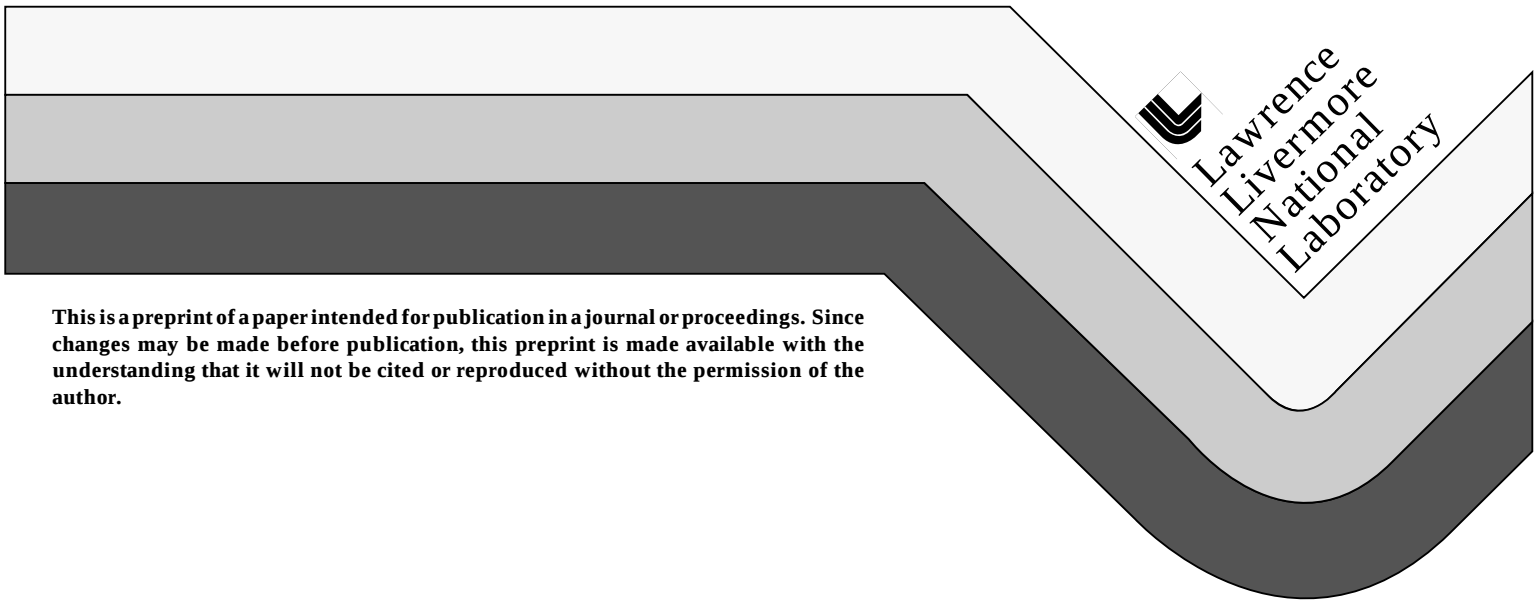

CHLOROFORM AND TRICHLOROETHYLENE UPTAKE FROM WATER INTO HUMAN SKIN *IN VITRO* : KINETICS AND RISK IMPLICATIONS

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Introduction

A model recently proposed by the U.S. Environmental Protection Agency (EPA) predicts that short-term dermal uptakes of organic environmental water contaminants are proportional to the square root of exposure time.[1,2] It has been shown[3] that the EPA model estimates non-steady-state cumulative short-term dermal uptake $R(t)$ as mL cleared per exposed cm^2 (i.e., normalized uptake in cm) by time t (e.g., for $t \leq 40$ min), from a solution with constant concentration, as

$$R(t) \approx K_p 10^{0.00305MW} \sqrt{\tfrac{2}{3} t \text{ hr}^{-1}} \quad , \qquad (1)$$

where MW is molecular weight ($\times$ mol/g) and K_p is a permeability coefficient estimated by EPA using a regression equation[4] involving MW and octanol/ water partition coefficients fit to steady-state permeability data for 93 organic chemicals.[5]

Because Eq. (1) reflects non-steady-state conditions expected during showering/bathing, it explicitly accounts for dose delivered post exposure due to subsequent systemic absorption of chemicals stored in skin, and thus has been noted to offer an improvement over application of steady-state approaches to dermal exposure assessment.[1,2] Nevertheless, the model appears to underestimate dermal uptake, based on very limited *in vivo* uptake data obtained primarily using human subjects.[3] To further assess this model, we examined *in vitro* dermal uptake kinetics for aqueous organic chemicals using accelerator mass spectrometry (AMS), which allows convenient and highly sensitive measurement of ^{14}C in biological samples.[6] Specifically, we examined the kinetics of *in vitro* dermal uptake of ^{14}C-labeled chloroform (CF) and trichloroethylene (TCE) from dilute (5-ppb) aqueous solutions using full-thickness human cadaver skin exposed for relatively brief periods ($\leq$1 hr). AMS measurements obtained were used to compare the proposed uptake model to a simpler pharmacokinetic model of chemical partition between water and skin that implies initial first-order uptake kinetics.

Materials and Methods

Chemicals and tissues

[^{14}C]-CF (5.0 mCi/mmol, >98% purity, ICN) and [1,2-^{14}C]-TCE (13.1 mCi/mmol, >98% purity, Sigma), were diluted in methanol to stock solutions (CF, 30 μCi/mL; TCE, 55 μCi/mL). ~20 cm^2 of full-thickness adult Caucasian abdominal cadaver skin (HS) was obtained at autopsy and stored frozen for 1-2 wk prior to use.

Dermal exposures

HS samples were each exposed in a closed-vial partition system to a premixed exposure solution of dilute aqueous CF or TCE (5.0 $\pm$ $\leq$0.20 μg/L, as stock solution diluted in distilled water, determined by liquid scintillation). Each 1.36-cm^2 HS disk was cork-bore cut and fitted in an Al-lined screw cap with epidermis facing out, and screwed onto an 8.75-mL glass vial containing exposure solution, a magnetic stir bar and no head space; 6 vials were inverted, stirred (430 rpm), and observed for 1, 5, 15, 20, 30 and 60 min, after which tissue from each was sampled. Simultaneous 60-min control experiments were done with HS in a vial containing only distilled water.

Each exposed HS sample was blotted dry, and 3-5 0.054-cm^2 cores were taken by punch, dislodged, and placed into separate, pre-cooled 6$\pm$50-mm glass tubes containing reactants used for AMS analysis via standard procedures.[6,7] Briefly, a small amount of CuO and pure Ag foil were added to each pre-baked sample tube prior to use. After adding a tissue core, each tube was flame-sealed within an evacuated 9$\pm$150-mm quartz tube and heated (650˚C, 3 hr) to oxidize tissue to CO_2. The CO_2 was transferred *in vaccuo* to quartz tubes containing reactants for graphitization, which tubes were heat-sealed and heated (650˚C, 3 hr) to catalytically reduce CO_2 to 1-2 mg of graphite + ^{14}C onto 5-10 mg of Co powder. The powder was placed in a Cs sputter source to generate 35-keV C^- ions, which were then accelerated in a tandem Van de Graaff electrostatic accelerator to 32.5 MeV. After momentum and energy selection, ^{14}C ions were counted in an ionization detector. ^{14}C in standards and in control samples (to assess contamination) were similarly analyzed. Increased ^{14}C in exposed tissue samples was expressed as net fmol ^{14}C per sampled tissue plug above corresponding control values, corrected to standards. Normalized uptake from each exposure solution was obtained by dividing fmol ^{14}C measured in each tissue sample by the solution's activity (fmol ^{14}C/mL).

Dermal Uptake Models & Data Analysis

AMS data obtained for *in vitro* uptake of CF and TCE into human skin from dilute aqueous concentrations were compared to corresponding uptakes predicted by a 1-compartment adaptation (Model 1) of a pharmacokinetic model of vapor uptake via skin.[8] According to Model 1, chemical concentration $C(t)$ in skin at time t is determined by uptake from an adjacent constant aqueous concentration C_w and from arterial blood, and by chemical loss back to the aqueous solution and into venous blood at concentration $B(t)$ exiting skin. Uptake and loss from skin are treated as first-order processes with corresponding rate constants $k_1 = K_p(DP_{sw})^{-1}$ and $k_2 = Q(VP_{sw})^{-1}$, respectively, such that

$$\frac{dC(t)}{dt} = k_1\left(C_w P_{sw} - C(t)\right) + k_2 P_{sb}\left(B_a - B(t)\right) \quad (2)$$

$$R(t) \approx \frac{K_p}{k_1 + k_2}\left(1 - e^{-(k_1+k_2)t}\right) \quad , \quad (3)$$

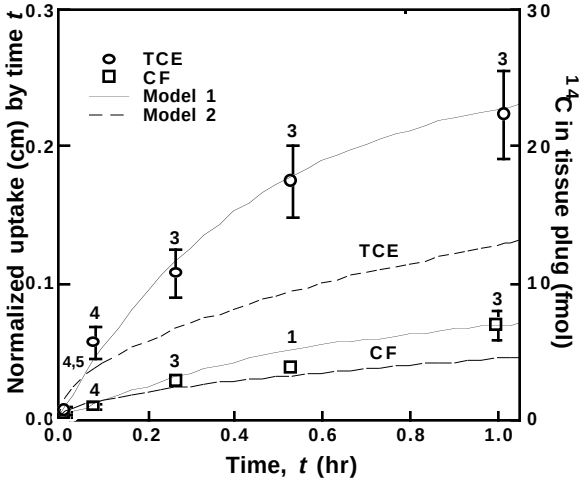
where Q is blood flow to skin, V is dermal volume, P_{sw} and P_{sb} are the skin/water and skin/blood partition coefficients, D is dermal depth, and B_a is arterial blood concentration. Under experimental *in vitro* conditions, $k_2 = Q = 0$, $[dR(t)/dt\,|t=0] = K_p$, $R(\infty) = K_p/k_1$, and $P_{sw} = R(\infty)D^{-1}$.

The ability of Model 1 to fit the AMS data was compared that of an EPA-type model (Model 2), namely, the right-hand expression in Eq. (1) in which K_p was fit specifically to data obtained in the present study (corresponding to *the best possible fit* to these data for an EPA-type model). K_p in Model 2 was estimated by weighted least-squares general-linear-model regression; K_p and k_1 in Eq. (3) ($k_2 = 0$, Model 1) were obtained by Levenberg-Marquardt X^2-minimization, using inverse sample variances as weights after testing for variance non-homogeneity using Bartlett's test; goodness-of-fit was assessed as the probability that X^2 is greater than a χ^2 distribution evaluated with df = (# data points) – 2.[9] P_{sw} was estimated assuming D = 0.0020 cm, a reasonable upper bound on *stratum corneum* depth.[10]

Results

AMS measures obtained for net uptakes of aqueous CF and TCE into human full-thickness skin *in vitro*, and corresponding model fits, are summarized in Figure 1. The mean absolute ^{14}C contents (± SD) of control tissue plugs were 0.30 ± 0.06 and 0.68 ± 0.13 fmol, respectively. Analysis of variance in net ^{14}C uptakes indicated significant heteroscedasticity in CF (χ^2 = 33.6, df = 4, $p < 10^{-6}$) and TCE (χ^2 = 14.4, df = 5, p = 0.013) uptake measurements, justifying use of weighted optimization procedures to obtain model fits Corresponding parameter estimates and goodness-of-fit statistics are summarized in Table 1. The figures and the latter statistics indicate clearly that the pharmacokinetic model (Model 1) is consistent with AMS uptake data obtained for both CF and TCE using human skin *in vitro*, and that the EPA-type model (Model 2) is not consistent with these data.

Figure 1. Uptake of dilute aqueous CF and TCE into full-thickness human cadaver skin.[a]



[a]Means of # values shown, ± 1 SD. Data fit to Model 1 (1st-order uptake) and Model 2 (EPA-type model[1,2]); normalized uptake = cm^3 water cleared per cm^2 exposed dermal area.

Discussion

The experimental measurements of *in vitro* CF and TCE uptake by human skin from dilute aqueous solution indicate that short-term, non-steady-state, dermal uptake kinetics for both compounds are consistent with a first-order partition model (Model 1). In particular, initial uptakes were found to be nearly linear, with approximate first-order decline in chemical uptake rate approaching a virtual zero-uptake rate estimable from data obtained using exposures of at least 1 hr. The estimated initial, normalized uptake rate (K_p) of 0.079 (± 7.4%) cm/hr for CF (Table 1) is fairly close to estimates of 0.11 to 0.13 cm/hr for CF based on *in vivo* uptake studies involving hairless guinea pigs and human subjects—in the latter case, after adjustment of calculated uptake to correspond to an 18-m^3 exposed surface area and to account for expected CF concentrations in shower-water in contact with exposed skin.[3,11-15] The estimated

Table 1. Model fits to *in vitro* AMS data on dermal uptake by human skin.

Compound	$R(t)$ model	K_p (cm/hr)	k_1 (hr^{-1})	P_{sw} (unitless)	χ^2	df	p-value
CF	1	0.0789 (±7.4%)	1.69 (±22%)	23 (± 3%)	18.5	14	0.19
CF	2	0.0129 (± 4.8%)	–	–	56.7	15	$<10^{-6}$
TCE	1	0.320 (±10%)	2.27 (±21%)	70 (±23%)	19.8	14	0.14
TCE	2	0.0345 (± 6.5%)	–	–	116.	15	~0

K_p value of 0.32 (± 10%) cm/hr for TCE (Table 1) is also close to, but significantly greater than, a corresponding estimate of 0.23 (± 17%) cm/hr obtained in an *in vivo* study using hairless guinea pigs.[11] In contrast, measured uptakes were found to differ significantly from values predicted by the EPA-type model as proportional to the square root of exposure time. After 0.1-hr exposures (corresponding, e.g., to a typical shower-exposure), Model 2 underpredicts our measured *in vitro* uptakes of CF and TCE into human skin by factors of about 4 and 2.5, respectively. AMS measures at each exposure duration in our study were obtained from one piece of exposed skin tissue, and thus do not reflect additional potential sampling variability, although the data reflect a temporal series of uptake measurements pertaining specifically to sets of relatively homogeneous samples of human skin tissue. With this caveat, our modeling results using *in vitro* AMS data appear to be consistent with other evidence from *in vivo* studies that the EPA model may tend to underestimate human dermal exposures to organic water contaminants such as CF and TCE.[3] Additional *in vitro* and *in vivo* studies with other compounds are needed to assess the adequacy of the EPA model for the wider range of water pollutants for which it was intended.

The P_{sw} estimates obtained for CF (23 ± 23%) and TCE (70 ± 23%) in the present experiment (Table 1) are consistent with the values predicted by the proposed estimators 0.70 log K_{ow} and 0.74 log K_{ow}. by EPA[1] and by Cleek and Bunge[2], respectively (yielding P_{sw} values 23.9 and 28.7 for CF, and 49.4 and 61.8 for TCE, respectively). Because our partition-coefficient estimation assumed that *stratum corneum* was the effective skin-compartment volume of distribution, it may be that these estimates reflect only apparent, short-term equilibria, and that chemical uptake might continue into full-thickness skin at a slow rate (relative to k_1) for many hours. However, data used for the present analysis, including exposures only ≤ 1 hr, were adequately modeled as a single first-order process. Data obtained using different tissue sections for longer exposure periods would be required to reliably estimate, e.g., a second, much smaller rate constant governing *in vitro* partition between epidermis and underlying dermal tissue. It is not clear that including such a second partitioning process in the *in vivo* pharmacokinetic model described in Methods would substantially alter or improve the ability of this model to predict *in vivo* human dermal uptake of volatile, lipophilic compounds like CF and TCE from aqueous solution. Blood perfusion to skin is likely to cause relatively rapid equilibration of chemical concentration within epidermis, and perhaps underlying dermal tissue as well. This is expected particularly in shower-related exposure scenarios, involving relatively high water temperatures known to elicit a 10- to 15-fold increase in local dermal blood perfusion rates.[16] We plan to test this hypothesis in future experiments involving AMS analysis of dermal microbiopsies obtained from exposed human volunteers.

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